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Marine Chemistry 92 (2004) 123–134

MARINE CHEMISTRY

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Amino acid carbon isotopic fractionation patterns in oceanic dissolved organic matter: an unaltered photoautotrophic source for dissolved organic nitrogen in the ocean?

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Received 4 November 2003; received in revised form 15 April 2004; accepted 30 June 2004
Available online 22 October 2004

Abstract

The transfer of dissolved organic carbon (DOC) and nitrogen (DON) out of the surface ocean where it is produced to storage in the ocean's interior creates one of the largest reservoirs of reduced carbon and organic nitrogen on earth. In nutrient-depleted surface waters of the oligotrophic ocean, dissolved nitrogenous material is of key importance as a source of fixed nitrogen for heterotrophic organisms. Recent work has increasingly indicated that, contrary to previous ideas, recalcitrant chemical structure is not the central factor underlying the preservation of DOC and DON, leaving the major preservation mechanisms largely unknown. We employ here a stable isotopic approach to examine the metabolic source and transformation signatures imprinted in carbon isotopic fractionation patterns of amino acids, which are the major components of both particulate and dissolved organic nitrogen that can be identified at the molecular level. Compound-specific isotopic signatures from central Pacific particulate and dissolved organic matter indicate a profound difference in processing histories between these two material pools. Sinking particles show a clear imprint of heterotrophic resynthesis and alteration, while the much larger and older dissolved pool retains an unaltered signature of photoautotrophic synthesis, even in samples from the abyssal ocean. In addition, $\delta^{13}C$ signatures of enantiomers of alanine (D vs. L) in dissolved materials are indistinguishable. This isotopic data, in light of previously observed abundant D-amino acids in oceanic DOM, suggests that autotrophic prokaryotes may be a main source for dissolved nitrogenous material preserved over long time scales in the sea. Taken together, our results suggest that dissolved organic nitrogen preservation is not predominantly linked to heterotrophic

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0304-4203/$ - see front matter
doi:10.1016/j.marchem.2004.06.021

reworking and resynthesis, but instead there exists a non-discriminating and rapid shunt, effectively removing recently formed autotrophic biomolecules from further recycling.

Keywords: Dissolved organic matter; Dissolved organic nitrogen; Particulate organic nitrogen; Amino acids; Carbon 13 isotopes; Organic matter preservation

Regional index: Pacific ocean; Equatorial

1. Introduction

The advected export of dissolved organic matter (DOM) from the surface ocean into the ocean's interior represents one of the Earth's major carbon flux pathways (Carlson et al., 1994). DOM accumulating in the ocean's interior persists over multiple oceanic mixing cycles and is 2000–4000 years old on average (Bauer and Druffel, 1992), representing a reservoir of reduced carbon comparable in magnitude to atmospheric CO_2. In oligotrophic regions, the nitrogenous component of DOM plays a particularly vital role in oceanic biogeochemical cycles. The biological inaccessibility of this large fixed nitrogen reservoir represents an important control on upper ocean cycles, and its advection to depth represents a "nitrogen pump" fundamental to closing oceanic nitrogen budgets (Jackson and Williams, 1985; Williams, 1995).

The chemical identity of dissolved nitrogenous material is key to understanding its sources, roles in oceanic food webs, and the mechanisms by which it cycles. However, the large majority of dissolved organic nitrogen (DON) still cannot be described at the molecular level (Bronk, 2002). Abiotic condensations to form complex molecules were thought to be a central explanation for this, and have long been the paradigm for interpreting long-term preservation of DOM in the sea. However, a growing body of evidence indicates that most organic material dissolved in the oceans is made up of intact biomolecules at all depths (Benner et al., 1992; Aluwihare et al., 1997; McCarthy et al., 1997), leaving the fundamental processes for this seemingly unlikely preservation largely unknown. Understanding the specific source organisms and processing histories of major classes of nitrogenous organic molecules may thus prove central to elucidating the processes controlling cycling and long-term preservation of the ocean's DON pool.

Amino acids (AA) account for most nitrogen in living organisms as well as the large majority of identifiable organic nitrogen in both DOM and particulate organic matter (POM). AA may also account for the majority of organic carbon exported to the ocean's interior via sinking particles (e.g., Hedges et al., 2001). In high-molecular weight DOM in particular, the predominance of the amide nitrogen functional group (McCarthy et al., 1997), coupled with low yields of amino sugars (Benner and Kaiser, 2003), suggest that AA comprise a majority of total DON, including that which cannot be recovered by standard hydrolytic methods. However, because AA molar ratios from diverse sources are similar, detailed information about AA source and processing from traditional molecular-level approaches has been limited.

We have employed here a powerful, under-utilized approach for tracing the origin and processing of detrital organic matter: the stable carbon isotopic fractionation patterns of individual AA (δ^{13}C-AA). Carbon and nitrogen isotopic fractionation in AA represents a direct record of the central metabolic cycles of an organism (Hayes, 2001). The major protein AA are synthesized by all microorganisms, however, pathways used for the synthesis of each can vary widely, resulting in unique patterns in their individual isotopic fractionations. Compound-specific measurements of both carbon (Keil and Fogel, 2001; Ziegler and Fogel, 2003) and nitrogen (McClelland and Montoya, 2002) AA isotopic distributions are now emerging as powerful tools for tracing metabolic sources and transformations in organic materials. The pathways for synthesis of AA carbon skeletons are more varied than for incorporation of nitrogen, thus while δ^{15}N patterns offer a uniquely detailed tracer for understanding trophic shifts (McClelland and Montoya, 2002), carbon isotopes can provide a more

detailed record of metabolic origin and resynthesis (Scott et al., submitted for publication). In contrast to most molecular-level tracers, δ^{13}C-AA is not linked predominantly to the phylogeny of a source organism, but rather to its central metabolic pathways. As a result, changes in relative δ^{13}C-AA patterns provide information regarding both metabolic source and specific transformation history. The inherent metabolic diversity of prokaryotes makes δ^{13}C-AA signatures particularly well suited to examine sources and microbial alteration of organic matter. Fortuitously for work with natural samples, δ^{13}C fractionations between individual AA are also very large relative to those observed in bulk organic matter. While fractionation between bulk compound classes is typically in the range of 1–5‰, fractionation between individual AA range up to 20‰ or even greater (Degens et al., 1968; Keil and Fogel, 2001; Macko et al., 1987).

We have examined δ^{13}C-AA patterns in particulate and dissolved material from the central Pacific, as well as in a suite of autotrophic and heterotrophic reference organisms. Our results indicate a dramatic bifurcation in processing history between particulate and dissolved organic materials, and suggest a surprising dominance of cyanobacterial sources for preserved deep oceanic DON. These observations lead us to propose a hypothesis for preservation of dissolved material in the oceans that may act largely independent of classic microbial-loop uptake and resynthesis.

2. Materials and methods

2.1. Samples

Large samples of high-molecular weight DOM were isolated by tangential flow ultrafiltration (UF) from four depths (2–4000 m) in the central Pacific Ocean (12°S, 135°W), after 0.1 μM pre-filtration to remove all particles and bacteria as described previously (Benner et al., 1997). Briefly, UF isolates 20–35% of the total DOM pool based primarily on molecular size, without chemical modification. Ultrafiltered DOM (UDOM) samples in this study were isolated using 1000 Da Amicon spiral-wound polysulfone membranes. Most UDOM chemical properties, including C/N, stable carbon isotopic ratios, radiocarbon 'ages,' overall amino acid content and AA D/L ratios, appear to be similar to the same properties for total DOM, and are clearly distinct from those of marine biomass or sinking particles (e.g., Benner et al., 1997; Guo et al., 1996; McCarthy et al., 1996). Because of the general similarities between properties of total DOM and UDOM, and the ease of using UF to isolate a significant fraction of the total DOC from seawater, UF has become widely used to examine seawater dissolved organics (Benner et al., 1992, 1997; McCarthy et al., 1996; Aluwihare et al., 1997; Guo, 2000). Our samples include material from both biologically active surface waters as well as abyssal waters free of any direct terrestrial influence, and represent some of the oldest DOM in the oceans (Bauer and Druffel, 1992). Sinking particles exiting the photic zone were also collected using floating rotating-sphere sediment traps from the same region on either side of the equator (1°S and 1°N, 135°W) at 105 m depth (Lee et al., 2000).

In addition to field samples, cultures of marine plankton and selected microbial heterotrophs were examined as examples of photoautotrophic and microbial heterotrophic sources. Cultures of widely distributed phytoplankton from diverse taxonomic groups (*Skeletonema costatum*, *Phaeocystis* sp., *Synechococcus bacillaris*) were grown in synthetic seawater media and harvested during exponential growth as described previously (Biddanda and Benner, 1997). *S. bacillaris* is a cyanobacterium of the family Cyanophyceae, the *Phaeocystis* sp. is a prymnesiophyte of the family Prymnesiophyceae, and *S. costatum* is a chain-forming pennate diatom of the family Bacillariophyceae. Two heterotrophic bacteria (*Shewanella oneidensis* and *Bacillus subtilis*) and one microzooplankton (*Brachionus plicatilis*) species were also examined. *S. oneidensis* (obtained from Dr. James Scott at Carnegie Institution of Washington) is a metabolically diverse bacterium widely distributed in oceanic and other environments, and broadly studied because of its ability to grow both aerobically as well as anaerobically using a diversity of electron acceptors. *B. subtilis* (obtained directly from Sigma) is found in various environments and is well studied in soils. *B. plicatilis* (obtained from Dr. Jay Brandes at the University of Texas) is a marine rotifer. The rotifers were raised at 32 ppt salinity and fed on submicron algae (J. Brandes, pers. comm.).

2.2. Amino acid hydrolysis and isotopic analysis

Individual AA isotopic analyses on UDOM and POM samples were made after acid hydrolysis (6 N HCl, 100 °C, 20 h) using isopropyl-TFA derivatives based on the method of Silfer (Silfer et al., 1991; Keil and Fogel, 2001). Samples were hydrolyzed in duplicate, derivatized, and analyzed on a Varian gas chromatograph coupled to a Finnegan Delta-Plus isotope ratio mass spectrometer (GC-IRMS). $\delta^{13}C$ values were measured for alanine (Ala), aspartic acid+asparagine (Asp), glutamic acid+glutamine (Glu), glycine (Gly), valine (Val), leucine (Leu), isoleucine (Ile), lysine (Lys), and phenylalanine (Phe). Most samples were chromatographed using a 50 m, 0.32 ID Hewlett Packard Ultra-1 column, while enantiomeric (D and L) determinations of Ala were run on a 25 m Alltech Chirasil-Val (0.32 ID) column. Total variability of individual $\delta^{13}C$-AA analyses was typically less than 1‰ for biological samples and ocean particles and 2–4‰ for UDOM. The higher variability in UDOM samples was due to the lower concentrations and much more complex chromatograms typical of this sample type, and varied depending on the relative concentration and chromatographic behavior of a given AA. For D and L-Ala, for example, analytical variability observed was at the low end of this range (Std. Dev. 1.2–1.9‰), because Ala is a major AA component and is chromatographically very well separated from other compounds. Because of higher analytical variability, four to five replicates of UDOM samples were run, while particulate and biological samples were analyzed two to three times.

To compare fractionation patterns of cultured organisms with environmental samples, each having unrelated carbon sources, the $\delta^{13}C$ values for each AA were normalized to the mean value (Figs. 1 and 2). This common statistical approach was done by subtracting the mean $\delta^{13}C$ value for all AA in a given sample from each individual AA $\delta^{13}C$ value. This approach moves the average $\delta^{13}C$-AA value to zero by shifting all individual values equally, and results in unaltered distribution patterns for each sample that now fall on a similar scale that can be readily compared. It is important to note that this normalization does not alter the relative magnitude of any isotopic shift and preserves the exact $\delta^{13}C$-AA pattern for all AA. Average $\delta^{13}C$ of all AA, rather than bulk organic matter, was chosen as the benchmark for this normalization due to the potential for unknown variation in composition between different sample types, with associated differences in overall $\delta^{13}C$ of different biochemical classes.

3. Results and discussion

3.1. Autotrophic and heterotrophic sources

We examined three reference photoautotrophs, the diatom *S. costatum*, a coccolithophore (*Phaeocystis* sp.), and a prokaryote (*S. bacillaris*). In each Ala, Asp and Gly was enriched in ^{13}C relative to the average, while Val, Leu, and Phe were generally depleted (Fig. 1a). The relative sense and magnitude of these shifts corresponds with autotrophic patterns observed previously, based on photoautotrophic AA biosynthesis (Abelson and Hoering, 1961; Hare et al., 1991; Macko et al., 1987). Importantly, with the possible exception of Gly (depleted in *S. bacillaris*), the relative fractionations of all three autotrophs were very similar, despite the fact that two are eukaryotes and one a prokaryote. This close correspondence underscores that relative $\delta^{13}C$-AA patterns reflect primarily central metabolic pathway, rather than source phylogeny.

Heterotrophic microbial biomass is characterized by different and much more variable $\delta^{13}C$-AA patterns than found in photoautotrophs (Fogel and Tuross, 1999; Keil and Fogel, 2001; Macko et al., 1987). This diversity is clear in the heterotrophic $\delta^{13}C$-AA patterns of two reference heterotrophs (Fig. 1b), which deviated substantially from the relatively tight autotrophic signatures (Fig. 1a). *B. subtilis* (Fig. 1b) displayed a $\delta^{13}C$-AA pattern common in many heterotrophic prokaryotes, with relative enrichment in Gly and Ala, and depletion in Ile, Val, and Leu (Keil and Fogel, 2001; Ziegler and Fogel, 2003). At the other extreme, *S. oneidensis* also differed markedly from the photoautotrophs; however, it displayed a more complex signature likely related to this organism's diverse metabolic abilities (Scott et al., submitted for publication). The exact patterns observed for a single organism are complicated by the fact that both uptake (salvage pathways) and

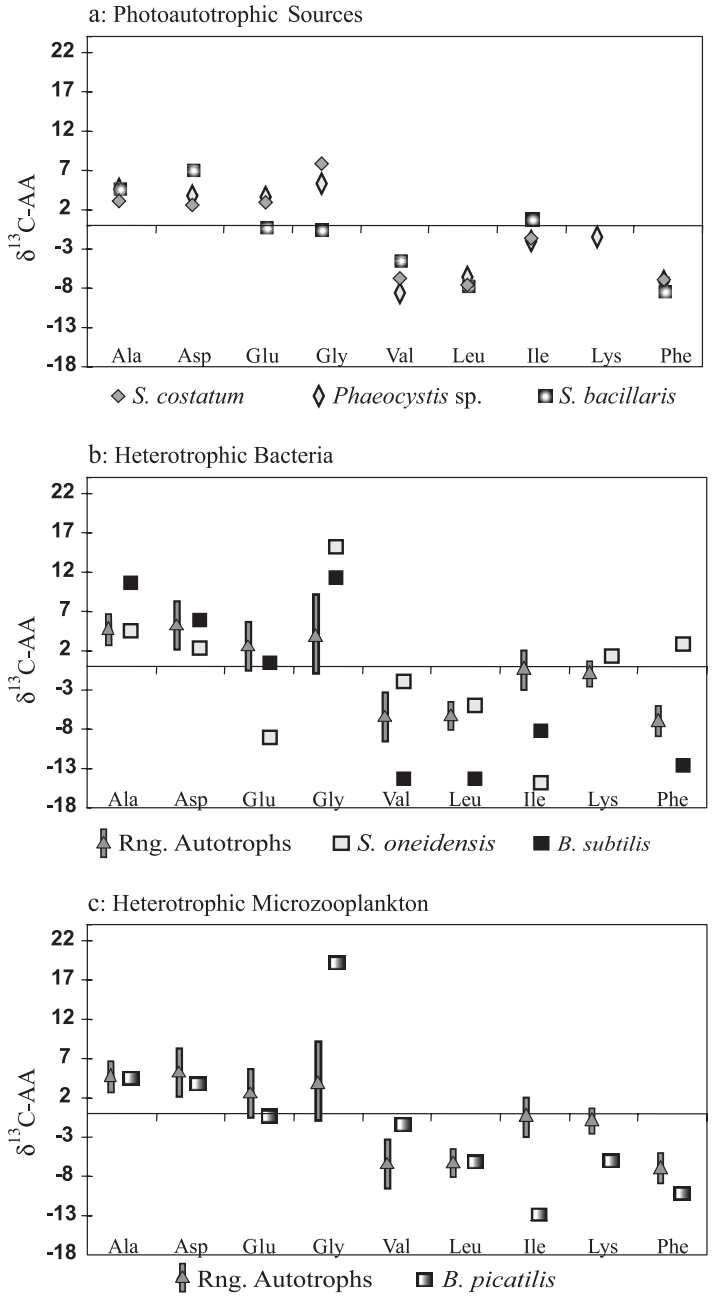

Fig. 1. δ^{13}C-AA signatures of autotrophic and heterotrophic plankton and microorganisms. Absolute δ^{13}C values are normalized to mean as described in Materials and methods, in order to plot on the same scale, allowing direct pattern comparison between samples. The same *y*-axis scale is used in all panels. (a) δ^{13}C-AA patterns for three photoautotrophic phytoplankton (*S. costatum*, *Phaeocystis* sp., *S. bacillaris*). Total span of δ^{13}C-AA values for all autotrophs is designated "Rng. Autotrophs" in subsequent panels. (b) δ^{13}C-AA patterns in microbial heterotrophs (*S. oneidensis*, and *B. subtilis*), compared to photoautotrophic source ranges. (c) δ^{13}C-AA patterns in a marine rotifer, *B. plicatilis*, relative to photoautotrophic source ranges.

Table 1
Individual δ^{13}C-AA fractionations for POM and UDOM isolates from the central Pacific

	UDOM					POM		
	mol%	2 m	100 m	375 m	4000 m	mol%	2N	1S
Ala	13	−17.5	−20.0	−21.6	−22.3	12	−16.1	−13.9
Asp	10	−18.5	−17.4	−16.3	−14.1	12	−17.4	−17.3
Glu	15	−17.8	−17.8	−16.8	−16.1	12	−16.0	−15.6
Gly	18	−13.6	−19.4	−18.4	−11.4	−13[a]	3.8	3.9
Val	3	−16.5	−23.9	−23.0	−22.4	7	−25.0	−24.6
Leu	4	−26.0	−23.7	−25.7	−21.4	9	−28.5	−25.8
Ile	5	−21.1	−24.0	23.9	−19.0	5	−32.2	−32.2
Lys	6	–	−16.0	−21.5	−13.5	–	−16.1	−18.4
Phe	1	–	–	–	–	–	−25.3	−26.1

Values are absolute δ^{13}C ratios for both UDOM and particles, and are not normalized as described in the text. Average molar percentages of individual AA (mol%) in each sample type are also shown for reference. Note that UDOM mol% showed no consistent trend with depth (McCarthy et al., 1996). Mol% in POM samples are also essentially identical between the two sites, with the exception of Gly as noted in the table (Lee et al., 2000). AA abbreviations are as defined in the text.

[a] Gly alone differed substantially between 2N and 1S: 16.9 mol% at 2N and 8.5 mol% at 1S.

resynthesis (de novo pathways) may occur for a given AA, in part depending on an organism's growing conditions. Thus in addition to greater heterotrophic variability, resynthesized AA should be most clearly and systematically altered, in particular if they were synthesized via a different metabolic pathway.

The largest and most ubiquitous shifts in our heterotrophs were ^{13}C depletion in Ile (7–13‰ relative to the average AA value) and ^{13}C enrichment in Gly (12–18‰). The ^{13}C depletion in branched AA, in particular Ile and Val, has been commonly observed to accompany microbial heterotrophic reworking (Fantle et al., 1999; Keil and Fogel, 2001), and has been attributed to the presence of the acetohydroxy acid synthetase pathway (Rawn, 1983). In our heterotrophs, depletion in both Val and Ile were observed; however, only Ile depletion was consistent in all samples. The strong Gly shifts also agree with previous observations suggesting that Gly ^{13}C enrichment may be the broadest δ^{13}C-AA indicator of heterotrophy (Fantle et al., 1999; Keil and Fogel, 2001). Elevated molar abundance of Gly has also been linked to heterotrophic processing in marine particles (Lee et al., 2000), sediments (Dauwe et al., 1999), and DOM (Yamashita and Tanoue, 2003), suggesting that Gly abundance and isotopic enrichment may be related indicators of heterotrophic reworking of organic matter in marine environments. The rotifer (Fig. 1c) had the most dramatic fractionation of the heterotrophs, but only in the two main markers Gly and Ile, with a 32‰ span between these two AA. Microzooplankton are major predators in aquatic ecosystems, consuming both algae and bacterial cells. It is not clear if the shifts in Ile and Gly are due mostly to de novo synthesis or to predation on bacterial sources; however, similar extreme Gly enrichments have been previously observed in ciliated protists (Ziegler and Fogel, unpublished data).

3.2. Sinking particles

Sinking POM samples showed similar patterns from both sites sampled, with relative fractionations of most AA indistinguishable from the photoautotrophic δ^{13}C-AA pattern in our reference organisms (Table 1; Fig. 2a). This tight concordance with the expected autotrophic signature confirms that δ^{13}C-AA patterns are clearly retained in oceanic detrital material. The main heterotrophic markers Gly and Ile are dramatic exceptions. Both these AAs showed very large deviations relative to the photoautotrophic pattern (+20‰ and −15‰, respectively, relative to the average), strongly indicative of major heterotrophic reworking. These sediment trap samples are from relatively shallow water (100 m), and were composed of a mixture of amorphous organic matter, diatoms, fecal pellets, and small heterotrophs such as foraminifera (Lee et al., 2000; Wakeham et al., 1997). While just at the base of the euphotic zone, previous molecular-level analyses have also indicated significant heterotrophic alteration by both bacteria and zooplankton (Lee et al., 2000). Because AA quantitatively dominate total organic matter in these particulate samples (Hedges et al., 2001; Lee et al., 2000), δ^{13}C-AA patterns likely represent a metabolic source-imprint for the majority of organic carbon leaving the surface ocean at the time of sampling. While specific source assignments are not possible, the very close correspondence to the rotifer pattern (Fig. 2b) may indicate that microzooplankton were a key source for sinking proteinaceous material, as suggested previously (Wakeham et al., 1997; Lee et al., 2000).

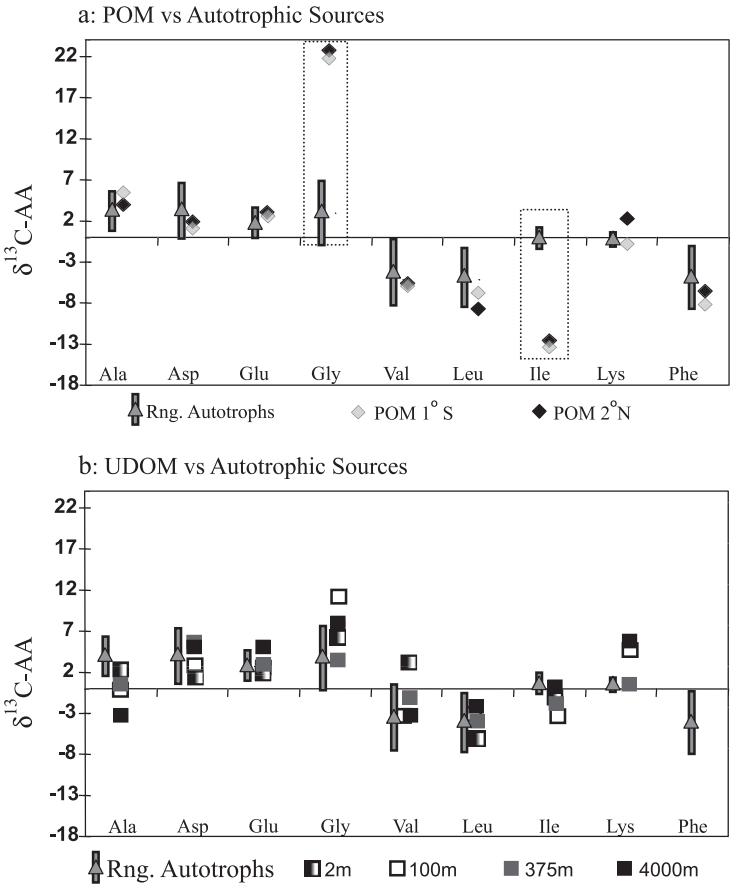

Fig. 2. δ^{13}C-AA signatures of central Pacific POM and UDOM. δ^{13}C-AA for sinking particles and UDOM isolates from the central Pacific. *Y*-axes are identical to Fig. 1. (a) δ^{13}C-AA patterns for particles exiting the photic zone (105 m) at 1°S and 1°N, 135°W. Range of photoautotrophic sources (Rng. Autotrophs) are shown for reference, as defined in Fig. 1. (b) δ^{13}C-AA patterns for UDOM from the water column in the central Pacific relative to photoautotrophic source ranges. Samples are from the surface (2 m), chlorophyll maximum (100 m), oxygen minimum (375 m), and the deep ocean (4000 m) at 12°S, 135°W.

3.3. Dissolved organic matter

In marked contrast to POM, UDOM AA signatures did not deviate substantially from autotrophic patterns at any depth in the central Pacific water column (Fig. 2b). While there was variability in AA isotopic shifts in the different UDOM samples (Table 1), it occurred within the analytical variation typical of the more complex DOM sample matrix (see Materials and methods), and was minor relative to the magnitude of heterotrophic shifts observed in reference materials or sinking POM (Figs. 1 and 2). Of the major heterotrophic AA markers, only Gly showed possible elevation at one depth (100 m), the biologically active zone where chlorophyll is at a maximum. There was no indication of a similar offset of Gly in the surface, oxygen minimum (375 m), or deep ocean (4000 m).

While trends too subtle to be observed in the current data set may exist, comparison to the large shifts typical of heterotrophically altered material strongly indicate that little heterotrophic resynthesis of dissolved THAA occurred in UDOM at any depth. The preserved autotrophic signature in deep ocean DOM is striking in part because the UDOM fraction has been found to have similar “old” radiocarbon ages as bulk DOM in both the Atlantic (Guo et al., 1996) and Pacific (McCarthy, unpublished data), indicating that deep UDOM has persisted over thousands of

years and multiple ocean mixing cycles. Dissolved organic matter in deep water has three major potential sources: advected transport from the surface, dissolution from sinking particles, and production by mid-water pelagic organisms. Advective transport to abyssal Pacific waters would result in very old material, while DOM from other pathways could be much younger. Because of the similarity in isotopic signal between surface and deep waters, and the difference between POC and DOC signals, particle dissolution is probably not a major source. Data on mid-water pelagic organism production is virtually non-existent. If the major source for our AA is thus mainly advective, proteinaceous material in deep DOM would be expected to be very old, and thus among the most intensively reworked of all active organic matter pools. The unaltered autotrophic δ^{13}C-AA signatures we observe suggest instead that most DON in the deep sea has not been heterotrophically recycled since initial formation in surface waters.

3.4. D-Amino acids and predominance of autotrophic bacterial source

Recent work has shown that oceanic DOM is characterized by high D/L ratios of selected AA, suggesting that an important component of DON is derived from prokaryotic sources (McCarthy et al., 1998; Fitznar, 1999; Amon et al., 2001). The autotrophic δ^{13}C-AA signatures in UDOM, given these high D-AA abundances, suggest that autotrophic prokaryotes (cyanobacteria) may be a dominant source for DON.

One way to examine this hypothesis is to directly measure the relative ^{13}C fractionation of D-amino acids, which are markers specific to bacterial biomass. If a major part of the autotrophic signal were in fact non-bacterial (i.e., derived from eukaryotic algae), then a difference in fractionation of the D vs. L enantiomers should result. In addition, if material in deeper waters increasingly derived from microbial heterotrophs, while surface input largely originated from autotrophs, one might also expect to see a divergence in signatures of D vs. L enantiomers with depth. Of the three most abundant D-AA in DOM (Ala, Gly, Asp) only D-Ala was abundant enough to examine the relative fractionation of D vs. L enantiomers under our analytical conditions. This is fortuitous, since among this group of AA Ala is most commonly observed to display an isotopic offset with heterotrophic processing (Ziegler and Fogel, 2003).

Relative fractionations of D vs. L-Ala were measured for UDOM at the surface and oxygen minimum, and found to be indistinguishable (Fig. 3). Comparison of values between 2 and 375 m are instructive because the surface represents the greatest relative abundance of newly produced autotrophic DOM, while the oxygen minimum represents a depth where large heterotrophic signal would be expected. The fact that at both of these depths the δ^{13}C of replicate analyses of D and L-Ala enantiomers are essentially identical, differing by less than 0.5‰, supports the interpretation of a preserved cyanobacterial source for both surface and deeper waters. It is important to bear in mind, however, that DOM in the two water masses likely have no direct temporal connection to each other, and likely derive from disparate regions of the world's oceans. This fact may underlie the observed ~2.5‰ difference in the absolute δ^{13}C values for both D- and L-Ala at the two depths. The close match between D and L enantiomers should also be investigated further, because the range of Ala fractionation in real ocean end-members is not known. Ala is an AA whose δ^{13}C shift reflects direct metabolic carbon supply, and thus the shifts associated with heterotrophic activity can be variable (Ziegler and Fogel, 2003). Work with larger samples, allowing isotopic measurements of all major D-AA, might provide a firmer interpretation.

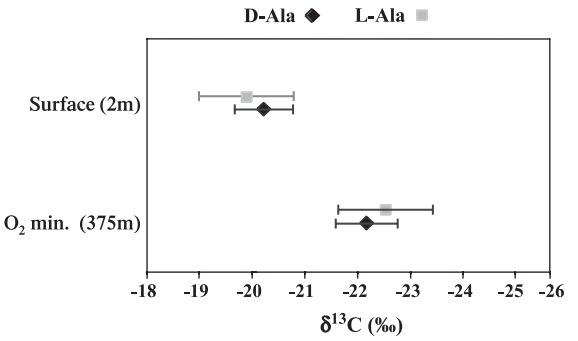

Fig. 3. δ^{13}C Amino acid values of Ala enantiomers (D- vs. L-Ala) from central Pacific UDOM. Enantiomeric D and L-Ala δ^{13}C values in UDOM from the surface (2 m) and oxygen minimum (375 m) depths. Note that values are not normalized, and ‰ scale (x-axis) greatly expanded relative to previous figures.

3.5. Dominance of cyanobacterial DON in the deep ocean?

While our data do not preclude contributions by autotrophic eukaryotes, they do suggest that eukaryotic material is a less important source of dissolved proteinaceous material reaching the deep ocean. This conclusion may be somewhat surprising, because heterotrophic grazing on eukaryotic phytoplankton is thought to be a principal pathway of DOM processing (Nagata, 2000). However, recent work by Nagata et al. (2003) has suggested that bacterial peptidoglycan generally cycles more slowly than protein. Thus a partial explanation may be that eukaryotic-derived algal material is degraded more rapidly, while prokaryotic material disproportionately accumulates in DOM. This interpretation is supported by the fact that higher proportions of Ala and Gly, both common components of peptidoglycan structures (Schleifer and Kandler, 1972), have been shown to correlate with decreasing THAA concentration (Dauwe and Middelburg, 1998; Yamashita and Tanoue, 2003), and thus are principle degradation indicators in a commonly cited AA degradation index ("DI", Dauwe et al., 1999). An autotrophic and prokaryotic origin for much THAA throughout the water column might also explain Yamashita and Tanoue's (2003) recent observation that in oceanic (as opposed to coastal) waters, no change in DI was observed with depth—despite the fact that overall THAA and DOM concentrations decreased as expected. If autotrophic bacteria are the major source of preserved THAA in open ocean regions, high abundance of bacterial markers might be linked more directly to this source than to subsequent degradation. Applying an index of degradation which works well in sediments (particle-derived material) or regions dominated by eukaryotic primary production (e.g., coastal or high latitude regions) may thus become more complicated in oceanic gyres where most primary production is prokaryotic.

Furthermore, when comparing different proxies for degradation the definitions used for specific pathways need to be clear. For instance, heterotrophic uptake with at least some de novo resynthesis, is one form of "degradation." Another form is exclusive heterotrophic catabolism, in which the remains of autotrophic food sources increase in abundance, but are never assimilated into heterotrophic biomass. If cell wall remnants from cyanobacteria were a disproportionate source of AA in oligotrophic regions, enrichment of this processed but not assimilated material would result in enrichment of the AA most abundant in cell walls, which should also retain the original autotrophic δ^{13}C-AA signatures.

The apparent dominance of autotrophic bacterial material throughout the water column is perhaps most surprising because it also suggests that heterotrophic bacteria, which are abundant everywhere in the sea, leave no discernable isotopic record of resynthesis in proteinaceous DOM. If the main pathways of preservation involved repeated passage through the microbial loop, then dissolved material surviving over long time scales might be expected to show the imprint of multiple cycles of heterotrophic bacterial uptake and resynthesis, with material in the deep sea representing the most extreme "heterotrophic" end member. Our δ^{13}C-AA data suggests that such a pathway is minor, even for the most labile compound classes such as amino acids. The bulk cellular and cell-wall makeup between autotrophic and heterotrophic bacteria are not vastly different, apart from light harvesting pigments and enzymes, suggesting little structure-based explanation as to why material from heterotrophic bacteria should be rare in preserved DOM.

The most direct explanation for predominance of cyanobacterial material may be linked to the relative magnitude of prokaryotic primary production in oceanic ecosystems. The central ocean basins are the largest biogeochemical provinces on earth (Karl, 2000), and in these areas primary production is dominated by prokaryotic picoplankton, with *Prochlorococcus* alone comprising up to 50% of the total (Partenski et al., 1999). If the major DOM preservation mechanisms are both rapid and relatively nonspecific, then δ^{13}C-AA signatures in DOM should primarily reflect relative magnitudes of source production. Heterotrophic microbial growth efficiency in oligotrophic surface waters is thought to be less than 20% generally, and possibly much lower in the deep sea (del Giorio and Cole, 2000). Thus, a direct extrapolation would suggest that the contribution of heterotrophic bacterial DOM might be an order of magnitude, or less, relative to that from autotrophic bacteria. Such a relatively small contribution, if not magnified by preferential preservation, might be easily obscured in δ^{13}C-AA signatures.

An alternate explanation for our isotopic results would be that the recycling of DOM by oceanic microorganisms does not appreciably alter δ^{13}C-AA, even though alteration in sinking particles does—thus resulting in the very different δ^{13}C-AA signatures in POM vs. DOM. This might be possible if microbial AA utilization in POM involved significant de novo synthesis of amino acids, while microbial activity in the dissolved phase utilized almost exclusively salvage pathways. Such an explanation, however, seems unlikely for the data set taken as a whole. In previous laboratory and field experiments, microbial heterotrophic assimilation (consumption and incorporation into biomass) clearly and substantially alters original AA fractionation patterns in DOM as well as particles (e.g., Ziegler and Fogel, 2003 and references therein). The very strong heterotrophic alteration signal in our sinking POM indicates that similar heterotrophic AA shifts are observed in the sea, in the same sense and magnitude that would be predicted from lab studies and reference organisms. There is little a priori reason to expect that such shifts would be associated with heterotrophic microbial activity only in particulate matter.

Another consideration is that the isotopic results reported here derive from the higher molecular weight fraction of DOM. While similar to total DOM in most respects, UDOM is also believed to be somewhat less diagenetically altered (Benner, 2002). Thus it is also possible that δ^{13}C-AA signatures might not extend in exactly the same way to lower molecular weight material. However, while sugar composition differs between UDOM and total DOM (Skoog and Benner, 1997), indicators of organic nitrogen quantity and quality (e.g., C/N ratios, THAA yields, D and L enantiomeric ratios) are very similar or indistinguishable between UDOM and total DOM pools (e.g., Dittmar et al., 2001; McCarthy et al., 1996). In addition, old radiocarbon ages of UDOM in the deep Pacific (~4000–5000 years; McCarthy, unpublished data) are very similar to those for total DOM (Bauer and Druffel, 1992), suggesting a similar cycling history for high and low molecular weight material. Taken together, it seems most likely that the δ^{13}C-AA patterns in UDOM do in fact indicate the predominance of autotrophic-sourced peptides and proteinaceous material throughout the subsurface ocean.

4. A hypothesis for DOM preservation

The apparent dominance of autotrophic sources for UDOM lead us to speculate that an important control on dissolved material preservation lies not in passage through repeated cycles of degradation and resynthesis, but instead takes the form of a "shunt" which rapidly protects a portion of newly produced upper-water biomolecules, resulting in eventual sequestration in the slowly cycling DOM reservoir. Such a mechanism would depend on very rapid processes in the surface ocean that could render even the most labile of dissolved materials, such as peptides and proteins, substantially less available for heterotrophic utilization. Recent laboratory experiments have suggested a number of possible mechanisms, including physical encapsulation into gel-matrix structures (Chin et al., 1998), hydrophobic protection as lipidic membrane fragments (Nagata et al., 1998), or action by "promiscuous" exoenzymes (Ogawa et al., 2001). Acting on a constant fraction of material produced from all sources, such a shunt could explain a predominance of autotrophic, and largely prokaryotic, AA in DOM throughout the water column. The similarity of δ^{13}C-AA patterns in both surface waters and abyssal DOM also suggests that some of the changes that occur are extremely robust, capable of lasting over ocean circulation time scales. Photochemical transformations may be among the most promising directions for research, as they can reduce the bioavailability of DOM and lead to refractory products (Benner and Biddanda, 1998). The implications of our results may also extend to other compound classes, as there is little reason to expect that such alterations would be confined to proteins and peptides, which are typically among the most rapidly cycled of all biochemicals (Cowie and Hedges, 1994). If true, understanding mechanisms for rapid and non-selective preservation of newly formed biosynthate may be a key challenge to understanding the long-term cycling of dissolved materials in the sea.

Acknowledgements

The authors gratefully acknowledge Jay Brandes, for providing the rotifer samples, James Scott for providing *S. oneidensis*, and Ben Van Mooy and Ian

Voparil for insightful discussions and suggestions. The Carnegie Institution of Washington, Geophysical Laboratory provided generous postdoctoral support for much of this work, and two anonymous reviewers also provided helpful suggestions. Greatest thanks are, however, to JIH. For everything. *Ad Astra per Alas Porci*.

References

Abelson, P.H., Hoering, T.C., 1961. Carbon and isotopic fractionation in formation of amino acids by photosynthetic organisms. Proc. Natl. Acad. Sci. 47, 623–632.

Aluwihare, L., Repeta, D.J., Chen, R.F., 1997. A major biopolymeric component to dissolved organic carbon in seawater. Nature 387, 166–169.

Amon, R., Fitznar, H.P., Benner, R., 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnol. Oceanogr. 46, 287–297.

Bauer, J.E., Druffel, E.R.M., 1992. ^{14}C activity of dissolved organic carbon fractions in the northcentral Pacific and Sargasso Sea. Nature 357, 667–670.

Benner, R., 2002. Chemical composition and reactivity of dissolved organic matter. In: Hansell, D., Carlson, C. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, pp. 59–90.

Benner, R., Biddanda, B., 1998. Photochemical transformations of surface and deep marine dissolved organic matter: effects on bacterial growth. Limnol. Oceanogr. 43, 1373–1378.

Benner, R., Kaiser, K., 2003. Abundance of amino sugars and peptidoglycan in marine particulate and dissolved organic matter. Limnol. Oceanogr. 48, 118–128.

Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I., Hatcher, P.G., 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. Science 255, 1561–1564.

Benner, R., Biddanda, B., Black, B., McCarthy, M., 1997. Abundance, size distribution, and stable carbon and nitrogen isotope compositions of marine organic matter isolated by tangential-flow ultrafiltration. Mar. Chem. 57, 243–263.

Biddanda, B., Benner, R., 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. Limnol. Oceanogr. 42, 506–518.

Bronk, D.A., 2002. Dynamics of DON. In: Hansell, D., Carlson, C. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, pp. 153–247.

Carlson, C.A., Ducklow, H.W., Michaels, A.F., 1994. Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea. Nature 371, 405–408.

Chin, W.-C., Orellana, M., Verdugo, P., 1998. Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature 391, 568–572.

Cowie, G.L., Hedges, J.I., 1994. Biochemical indicators of diagenetic alteration in natural organic matter mixtures. Nature 369, 304–307.

Dauwe, B., Middelburg, J.J., 1998. Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. Limnol. Oceanogr. 43, 782–798.

Dauwe, B., Middelburg, J.J., Herman, P.M.J., Heip, C.H.R., 1999. Linking diagenetic alteration of amino acids and bulk organic matter reactivity. Limnol. Oceanogr. 44, 1809–1814.

Degens, E.T., Behrend, M., Gotthard, B., Reppmann, E., 1968. Metabolic fractionation of carbon isotopes in marine plankton, II. Data on samples collected off the coasts of Peru and Ecuador. Deep-Sea Res. II 15, 11–20.

del Giorio, P., Cole, J., 2000. Bacterial energetics and growth efficiency. In: Kirchman, D.L. (Ed.), Microbial Ecology of the Oceans, Wiley Series in Ecological Science. Wiley-Liss, New York, pp. 289–326.

Dittmar, T., Fitznar, H.P., Kattner, G., 2001. Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. Geochim. Cosmochim. Acta 65 (22), 4102–4114.

Fantle, M., Dittel, A.I., Schwalm, S.M., Epifanio, C.E., Fogel, M.L., 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120 (3), 416–426.

Fitznar, H.P., 1999. D-Amino acids as tracers for biogeochemical processes in the river–shelf–ocean-system of the Arctic. Alfred-Wegmer Inst. Rep. Polar Res. 334, 116.

Fogel, M.L., Tuross, N., 1999. Transformation of plant biochemicals to geological macromolecules during early diagenesis. Oecologia 120, 336–346.

Guo, L., 2000. Re-examination of cross-flow ultrafiltration for isolating colloidal macromolecular organic matter in seawater. Mar. Chem. 69, 75–90.

Guo, L., Santschi, P.H., Cifuentes, L.A., Trombore, S.E., Southon, J., 1996. Cycling of high-molecular-weight dissolved organic matter in the Middle Atlantic Bight as revealed by carbon isotopic (^{13}C and ^{14}C) signatures. Limnol. Oceanogr. 41 (6), 1242–1252.

Hare, E.P., Fogel, M.L., Stafford, T.W., Mitchell, A.D., Hoering, T.C., 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J. Archaeol. Sci. 18, 277–292.

Hayes, J.M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. In: Cole, D.R., Valley, J.W. (Eds.), Stable Isotope Geochemistry. Reviews in Mineralogy and Geochemistry. Mineralogical Society of America, Washington DC, pp. 225–278.

Hedges, J.I., Baldock, J.A., Gelinas, Y., Lee, C., Peterson, M.L., Wakeham, S.G., 2001. Evidence for non-selective preservation of organic matter in sinking marine particles. Nature 409, 801–804.

Jackson, G.A., Williams, P.M., 1985. Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling. Deep-Sea Res. 32 (2), 223–235.

Karl, D.M., 2000. A sea of change: biogeochemical variability in the North Pacific subtropical gyre. Ecosystems 2, 181–214.

Keil, R.G., Fogel, M.L., 2001. Reworking of amino acids in marine sediments: stable carbon isotopic composition of amino acids in sediments along the Washington coast. Limnol. Oceanogr. 46, 14–23.

Lee, C., Wakeham, S.G., Hedges, J.I., 2000. Composition and flux of particulate amino acids and chloropigments in equatorial Pacific seawater and sediments. Deep-Sea Res. I 47, 1535–1568.

Macko, S.A., Fogel, M.L., Hare, P.E., Hoering, T.C., 1987. Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. Chem. Geol. 65, 79–92.

McCarthy, M.D., Hedges, J.I., Benner, R., 1996. Major biochemical composition of dissolved high-molecular weight organic matter in seawater. Mar. Chem. 55, 281–297.

McCarthy, M.D., Hedges, J.I., Pratum, T., Benner, R., 1997. Chemical composition of dissolved organic nitrogen in the ocean. Nature 390, 150–154.

McCarthy, M.D., Hedges, J.I., Benner, R., 1998. Bacterial origin of major fraction of dissolved organic nitrogen in the sea. Science 281, 231–233.

McClelland, J.W., Montoya, J.P., 2002. Trophic relationships and the nitrogen isotopic composition of amino acids. Ecology 83, 2173–2180.

Nagata, T., 2000. Production mechanisms of dissolved organic matter. In: Kirchman, D.L. (Ed.), Microbial Ecology of the Oceans, Wiley Series in Ecological Science. Wiley-Liss, New York, pp. 121–152.

Nagata, T., Fukuda, R., Koike, I., Kogure, K., Kirchman, D.L., 1998. Degradation by bacteria of membrane and soluble protein in seawater. Aquat. Microb. Ecol. 14, 29–37.

Nagata, T., Meon, B., Kirchman, D.L., 2003. Microbial degradation of peptidoglycan in seawater. Limnol. Oceanogr. 43, 745–754.

Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of refractory dissolved organic matter by bacteria. Science 292, 917–920.

Partenski, F., Hess, W., Vaulot, D., 1999. *Prochlorococcus*: a marine photosynthetic prokaryote of global significance. Microbiol. Mol. Biol. Rev. 63 (1), 106–127.

Rawn, D.J., 1983. Biochemistry. Harper and Row. 1139 pp.

Schleifer, K.H., Kandler, O., 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriological Revs. 36, 407–477.

Scott, J.H. et al., submitted for publication. Examination of isotopic effects associated with amino acid biosynthesis in microbes. Proc. Natl. Acad. Sci.

Silfer, J.A., Engel, M.H., Macko, S.A., Jumeau, E.J., 1991. Stable Carbon isotope analysis of amino acid enantiomers by conventional isotope ratio mass spectrometry and combined gas chromatography/isotope ratio mass spectrometry. Anal. Chem. 63, 370–374.

Skoog, A., Benner, R., 1997. Aldoses in various size fractions of marine organic matter: implications for carbon cycling. Limnol. Oceanogr. 42, 1803–1813.

Wakeham, S.G., Hedges, J.I., Lee, C., Peterson, M.L., Hernes, P.J., 1997. Compositions and transport of lipid biomarkers through the water column and surficial sediments of the equatorial Pacific Ocean. Deep-Sea Res. II 44, 2131–2162.

Williams, P.J.L.B., 1995. Evidence for seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. Mar. Chem. 51, 17–29.

Yamashita, Y., Tanoue, E., 2003. Distribution and alteration of amino acids in bulk DOM along a transect from bay to oceanic waters. Mar. Chem. 82, 145–160.

Ziegler, S., Fogel, M.L., 2003. Seasonal and diel relationships between the isotopic compositions of dissolved and particulate organic matter in freshwater ecosystems. Biogeochemistry 64, 25–52.